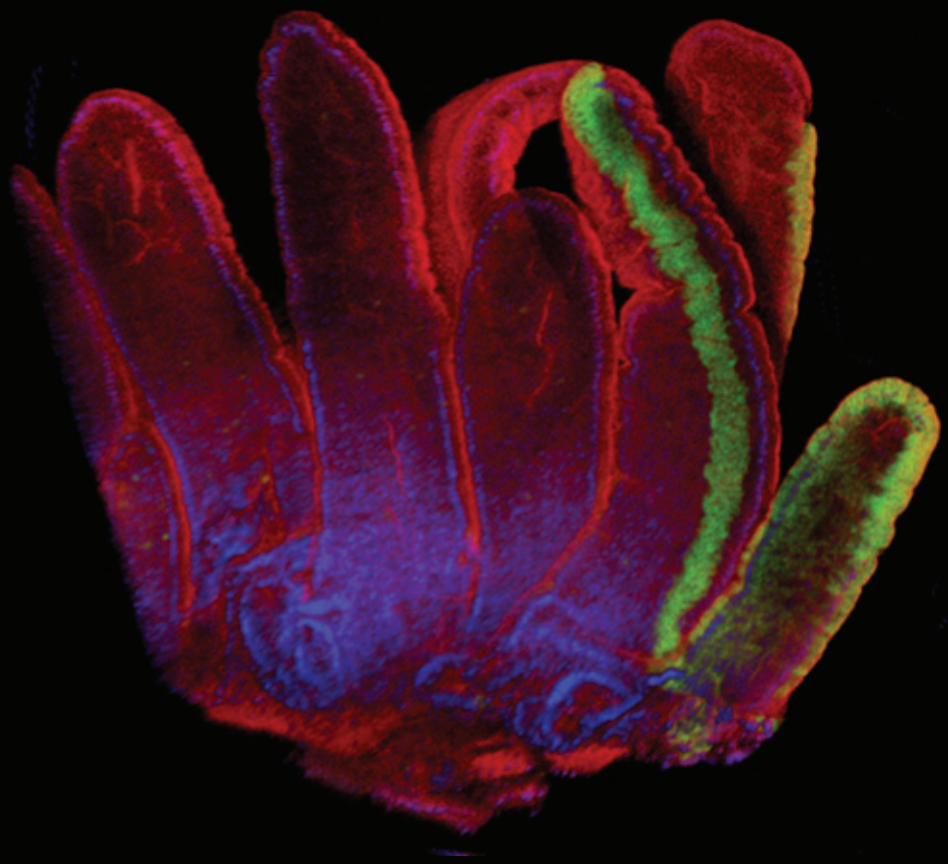

Intestinal stem cells from a specific lineage (green) travel up finger-like projections called villi on the surface of the mouse intestine to replace cells that were lost following injury from radiation. This type of stem cell complements the functions of other types of stem cells present in the intestine, which are involved in repopulation during normal cell turnover. This image was captured using a technique called three-dimensional confocal reconstruction, which allows the visualization of gene expression.

Image courtesy of Dr. Manuel Amieva, Dr. Calvin Kuo, and Dr. Kelley Yan, Stanford University.

Digestive Diseases and Nutrition

Digestive diseases are among the leading causes of doctor visits, hospitalizations, and disability in the United States each year. These conditions span a wide spectrum of disorders that affect the gastrointestinal (GI) tract, liver, gallbladder, and pancreas, as well as obesity and other nutrition-related disorders. In 2004, more than 35 percent of all emergency and outpatient hospital visits—some 100 million—were associated with a diagnosis of a digestive disease.[1] While some digestive diseases are common and others quite rare; collectively, they exact a significant toll on public health in terms of their effects on quality of life, years lost due to premature death, and costs associated with hospitalization and pharmaceutical and surgical interventions. To reduce the public health burden associated with digestive diseases, NIDDK-supported scientists are vigorously pursuing research to better understand how widespread these diseases are across the United States and in specific population groups, to identify the causes of these diseases and how they progress, and to test new interventions for prevention and treatment of these costly diseases, including drugs, surgery, and behavior modification.

Inflammatory bowel diseases (IBD), which include Crohn's disease and ulcerative colitis, are marked by destructive inflammation in the intestinal tract leading to rectal bleeding, diarrhea, nutritional deficiencies, and other serious complications. These diseases often strike early in life, with a peak age of onset in adolescence or young adulthood. Treatment may require surgery, including removal of the affected region of the intestine. Scientists are investigating the complex interactions among the genetic, environmental, immune, microbial, and cellular factors that contribute to the development of IBD. The continued discovery of predisposing genetic variations, potential autoimmune and microbial influences, and new methods to grow intestinal tissue in cell culture will help catalyze the design of novel therapeutic strategies. Research on controlling intestinal inflammation has potential benefits not only for patients with IBD, but also for those at risk of developing colorectal cancer.

Diseases of the stomach and intestines include some of the most common digestive diseases, such as peptic ulcer disease, which is typically caused by an infection with the bacterium *Helicobacter pylori*, or use of non-steroidal anti-inflammatory drugs. Stomach and intestinal disorders also include functional bowel disorders, which result in abdominal pain and altered bowel habits. For example, irritable bowel syndrome (IBS) causes pain and constipation or diarrhea. IBS more frequently affects women, who may display a different range of symptoms and respond differently from men to pharmacologic treatments for the disease. While diet and stress contribute to this disorder, its underlying causes are unknown. Gastroesophageal reflux disease, in which stomach acids rise up into the esophagus, is a common functional bowel disorder that can lead to a condition known as Barrett's esophagus. This condition, in which cells lining the esophagus turn into an intestinal type of cell, is associated with a heightened risk of esophageal cancer, which is one of the cancer types still on the rise in the United States. Gastroparesis is another

[1] *Everhart JE, editor. The burden of digestive diseases in the United States. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases. Washington, DC: U.S. Government Printing Office, 2008; NIH Publication No. 09-6443.*

functional bowel disorder, which is characterized by delayed emptying of food from the stomach, resulting in nausea, vomiting, and abdominal discomfort. A common cause of gastroparesis is diabetes, which is thought to damage nerves leading to the stomach and controlling movement of food; however, many cases are of unknown origin. Fecal incontinence, or impaired bowel control, is another bowel disorder that poses a major public health burden, particularly in the elderly.

Some digestive diseases can be triggered by the body's reaction to certain foods. For example, in individuals with celiac disease, the immune system reacts to the protein gluten—a component of wheat, barley, and rye—and damages the small intestine. This damage interferes with the ability of the intestine to absorb nutrients from foods and can result in chronic diarrhea, bloating, anemia, and, in children, slower growth and short stature. The only current treatment for celiac disease is maintenance of a strict gluten-free diet, which is difficult for many people. The greater challenge now facing patients and their health care providers is to improve methods capable of diagnosing celiac disease early, before damage occurs or other conditions develop. Recent and continued advances in the understanding of genes that predispose individuals to develop celiac disease may contribute to improved diagnosis in the future through genetic-based screening.

The microorganisms that inhabit the GI tract are important factors in maintaining or tipping the balance between digestive health and disease. These microbes can affect intestinal health in some surprising ways, depending on their interactions with each other, with intestinal cells, and with nutrients ingested by their human host. Scientists are gaining insights into the ways these GI microorganisms influence the development and function of the digestive tract and other systems throughout the body, such as those with immune and metabolic functions, as well as how the composition of the GI microbial community changes with factors such as age, geography, diet, and antibiotic usage.

The exocrine pancreas, which secretes enzymes required for digestion, is vulnerable to disorders such as acute and chronic pancreatitis, and their complications. Common causes of pancreatitis may include gallstones, heavy alcohol use, inherited genetic factors, drugs, and other causes. In all forms of pancreatitis, digestive enzymes attack the pancreas from within, causing inflammation, loss of function, and severe pain. Research has elucidated genetic and other factors contributing to pancreatitis that may lead to ways to treat or prevent this disorder.

The liver is an organ within the digestive system that performs many critical metabolic functions, including processing and distribution of nutrients such as fats. When the liver is functionally compromised by disease, serious adverse effects on health can occur, which sometimes lead to complete liver failure. Some liver diseases primarily affect children, such as biliary atresia (a progressive inflammatory liver disease), while others generally affect adults, such as a form of nonalcoholic fatty liver disease (NAFLD) or nonalcoholic steatohepatitis (NASH). In recent years, however, NAFLD has been increasingly diagnosed in children in the United States as well, concurrent with rising overweight and obesity. While some forms of liver disease are caused by viral infection, such as hepatitis B and C, or by genetic mutations such as alpha-1 antitrypsin deficiency, others arise from diverse factors such as autoimmune reactions, drug toxicity, and other triggers, some of which are unknown. Many liver diseases, such as chronic hepatitis B and C, place individuals at elevated risk for developing liver cancer. A healthy liver is necessary for life, and the only treatment for end-stage liver disease is a liver transplant. Because the number of livers available from deceased donors is limited, research is critical to identify liver disease early, find methods to preserve liver function in people with liver disease, and develop new treatment options, including transplants performed with liver tissue from living donors.

The number of Americans who are overweight or obese has risen dramatically in recent decades and is now at epidemic levels. Obesity is associated with numerous diseases, including type 2 diabetes, heart disease, and cancer. Multiple factors contribute to obesity. As scientists elucidate the molecular, genetic, and environmental factors that influence

appetite, metabolism, and energy storage, they are identifying potential avenues for the development of new intervention strategies to promote safe, long-term weight loss. In addition to new pharmacologic interventions for obesity, existing bariatric surgical techniques are being evaluated for their long-term impacts on weight loss and well-being. Investigators are also continuing research to help people achieve healthy lifestyles that include physical activity and improved diet. (Additional information on NIDDK-supported research endeavors focusing on obesity is provided in the Obesity chapter.)

Other nutrition-related disorders under investigation involve specific, inherited alterations in nutrient metabolism. NIDDK-supported research has enhanced knowledge of how these nutritional disorders develop, and how they can best be treated.

NEW INSIGHTS ABOUT GUT BACTERIA AND HUMAN HEALTH

Long-term Diet Determines Which Bacteria Reside in the Gut: In their quest for greater understanding of how microbes inhabiting the body affect human health and wellness, scientists have found an association between individuals' long-term diets and the microbes that predominate inside their intestines. The current study analyzed the community of bacteria (microbiota) that populate the intestine and the effect of diet on different types of bacteria. For the first study, designated as "COMBO," a group of 98 healthy volunteers were asked to complete 2 questionnaires—1 asking for information on their diets over a long period of time (Food Frequency Questionnaire), and another asking the volunteers what they had eaten recently (Recall Questionnaire). The volunteers also provided stool samples, which contained bacteria from the intestines. The scientists then assessed the nutrients consumed by each of the volunteers by analyzing the dietary information from the questionnaires, and they identified the bacterial species within the gut microbiota by sequencing the bacterial DNA from the stool samples. Analyses of the DNA showed the gut microbiota of the volunteers contained two primary clusters of bacterial types found in the intestine, known as enterotypes—the *Bacteroides* enterotype and the *Prevotella* enterotype—with one of the two identified as dominant for each volunteer. By comparing dietary nutrients and bacterial species, the scientists determined that long-term dietary composition was associated with enterotypes. The *Bacteroides* enterotype was highly associated with a diet high in animal protein and saturated fat, whereas the *Prevotella* enterotype was associated with a high-carbohydrate diet. Ten of the volunteers also participated in a study called "CAFE," in which they ate a controlled diet while living in a hospital environment. All of the CAFE participants had been identified in the COMBO study as having dominant *Bacteroides* enterotypes, associated with diets high in animal protein and saturated fat. Half of the volunteers were given the high-fat/low-fiber (*Bacteroides*) diet, and half were given a low-fat/high-fiber (*Prevotella*) diet. Changes in microbiota composition were seen within 24 hours of starting a new type of diet, as detected by changes in the collective microbial genomes. Although significant, none of these changes in microbiota composition resulted in lasting changes in the volunteer's enterotype during the 10 days of the CAFE study. These studies indicate that, while dietary components over the short-term affect gut bacterial populations, long-term dietary composition determines the dominant enterotype of bacteria in the human intestine. This research has demonstrated an association between long-term diet and gut bacterial enterotypes. If future research finds that gut enterotypes, like dietary patterns, are associated with particular diseases, these results could have important implications for treating diseases through long-term dietary interventions to produce a healthy gut enterotype.

Wu GD, Chen J, Hoffmann C, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science 334:105-108, 2011.

The Intestinal Microbiome Revealed as a Source of Human Genetic and Metabolic Diversity: A recent study found that the composition of bacterial species that populate the human gut—the gut microbiota—evolves with age, particularly in the first years of life, and it differs among people from diverse geographic regions, potentially reflecting varying nutrition. Scientists

sequenced the gut microbiomes (microbiota DNA) of healthy individuals of different ages from the Amazonas of Venezuela, the African nation of the Republic of Malawi, and metropolitan areas of the United States to determine whether differences could be discerned in the diversity of bacterial communities and in the metabolic and nutritional functionality of the genes they contained. Microbiome DNA was obtained from fecal samples donated by members of the study cohort, which included parents, children, siblings, and identical and fraternal twins. A broad spectrum of information was obtained from the analysis of the microbiome data. Of particular importance, microbiota bacterial diversity increased with age in all populations, and bacterial species composition evolved from an infant microbiota into an adult microbiota during the first 3 years of life. In addition, the repertory of microbiome genes involved in vitamin biosynthesis, carbohydrate metabolism, and other metabolic functions also changed with age and differed among the countries. There were greater differences in bacterial community composition among the children than among the adults, and there were significant differences in the types of bacteria represented by the microbiomes of the three geographically representative populations. The greatest differences among populations were seen between the United States and the other two countries, in terms of their bacterial capacities for metabolizing vitamins, carbohydrates, proteins, and other substances, which closely reflect dietary patterns in these countries. This study reveals significant differences in the gut microbiome among young children and adults and among cultures with different diets, underscoring the importance of considering microbiome contributions in studies and nutrition-related policies involving human development, nutrition, physiology, and the impact of westernization.

Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. Nature 486: 222-227, 2012.

"Friendly" Bacteria Compete with Disease-causing Bacteria in the Intestine: In health, trillions of microbes (the microbiota) inhabiting the human gastrointestinal tract co-exist with their host in a relationship that is beneficial for both host and microbiota. However, when disease-causing (pathogenic) bacteria invade the gastrointestinal tract, they can disrupt this symbiotic relationship. Research scientists have recently uncovered important events that occur during pathogen infection.

Certain types of *E. coli* bacteria cause severe diarrhea when they infect the intestine. In a study exploring the invasion of hosts by virulent bacteria, research scientists used mice that were infected with a type of bacteria that mimic human *E. coli* infection, as a model system of the infection process. Intestinal infection requires that the invading pathogen compete with resident bacteria of the gut for nutrients and space. The symbiotic residential bacteria, known as commensals, make their home in the surface layer of the intestine called the outer mucosa. When pathogens invade, they begin synthesizing and secreting virulence factors that disrupt the outer and inner mucus layers of the intestine. Burrowing through the damaged mucosa, the pathogens colonize the unoccupied niche of intestinal epithelial cells where they cause damage and inflammation. In this study, the researchers infected different groups of mice with the pathogenic bacteria; one group had been raised in the absence of normal bacteria (germ-free) prior to infection, and the other group had been conventionally raised. They found that the conventionally raised mice were able to clear the infection, but the mice raised germ-free were not. Also, analysis of pathogen virulence factors revealed that they are important in conventionally raised mice for the early stage of infection, but production of these factors diminished later in infection, forcing the pathogens from their cellular niche back into the intestine's mucosa. There, the pathogens must compete with the commensals for a diet of simple sugars that they, the pathogens, require. However, in this environment the commensals tend to have the competitive advantage. Commensals that grow best on simple sugars successfully compete with the pathogens for this food. In fact, previous research found that infection by these pathogens alters the microbial community to promote growth of this type of commensals; the current study shows that these commensals then provide the benefit of devouring the food needed by the pathogens. Other types of commensals that are less picky about which

sugars they will eat do not out-compete the pathogens when mice are fed a normal diet of multiple types of sugars, but when faced with a diet of only simple sugars, they too will compete for this food, starving the pathogens and clearing infection. The knowledge that infection and clearance of intestinal pathogens is the result of both expression of virulence factors and a competition for nutrients indicates that diet or probiotic approaches may be explored for future treatment of intestinal infections.

Kamada N, Kim Y-G, Sham HP, et al. Regulated virulence controls the ability of a pathogen to compete with the gut microbiota. Science 336:1325-1329, 2012.

Diet High in Milk Fat May Promote Harmful Intestinal Bacteria and Inflammation: Scientists have shown that mice with a pre-existing genetic susceptibility to intestinal inflammation fed a diet high in saturated fats from milk have altered intestinal microbial communities that occur along with changes in bile acid composition, altered immune function, and increased intestinal inflammation. Inflammatory bowel diseases (IBD), including Crohn's disease and ulcerative colitis, are thought to result from a complex interplay between genetic and environmental factors. The rising incidence of these conditions in recent decades, together with studies showing increases in IBD in immigrants relocating from low-prevalence to high-prevalence countries and their children, point to a growing influence of environmental factors, such as diet. The dynamic communities of intestinal microbes, which are profoundly interdependent with humans in terms of metabolizing dietary nutrients, have received more attention in recent years for their potential contributions to human health and diseases such as IBD. In this study, scientists fed mice for 3 weeks on a diet high in fat—either milk fats, lard, or safflower oil from plants—which mimicked the fat levels found in Western diets, and compared them to mice fed a low-fat diet. They first looked to see the effect of these diets on the types and abundances of microbes present in the stool using genetic sequencing. All of the high-fat diets reduced the diversity of microbes present compared to the low-fat diet. In mice fed the high milk-fat diet, the scientists observed a "bloom" or explosion in the number of a particular bacterium, called *Bilophila wadsworthia*—a bacterium often detected in illnesses such as appendicitis and other types of intestinal inflammation. The researchers also examined mice with a genetic susceptibility to develop intestinal inflammation (colitis) due to deficiency of the gene encoding *Il10*, which is part of the immune system. These mice showed increased colitis when fed a milk-fat diet compared to the susceptible mice fed diets high in fats from plants or low in fat, or compared to mice without the genetic risk that were fed the high milk-fat diet. The genetically susceptible mice fed high milk-fat also had altered immune functions and more abundant levels of *B. wadsworthia* and taurocholic acid, a form of bile acid in which these bacteria thrive. Of note, the byproducts of bacterial metabolism of these bile acids and other substrates can injure and breach the inner protective barrier of the gut, leading to inflammation and damage. This pioneering work in mice connects the dots between genetics, the immune system, diet, and microbes to outline a compelling picture of how these factors may be interacting in the development of human intestinal inflammatory conditions such as IBD. While these findings require replication in humans, they offer a glimpse into the future of how these diseases might be treated or prevented in susceptible individuals through dietary and/or microbial means.

Devkota S, Wang Y, Musch MW, et al. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10-/- mice. Nature 487: 104-108, 2012.

Early Exposure to "Friendly" Microbes Protects Against Inflammatory Bowel Disease (IBD) and Asthma: Diseases such as IBD and asthma are believed to occur, in part, because of inappropriate immune responses to "friendly" bacteria. However, exposure early in life to these bacteria appears to modify disease responses. In a study designed to explain these apparent contradictions, scientists have used mouse models to detect the relationships of microbes and immune cells to IBD and asthma. The scientists began their study by looking for pro-inflammatory cells called iNKT (invariant natural killer T) cells in tissues that line the intestines and

lungs of mice. These iNKT cells have been associated with inflammation related to ulcerative colitis (UC), a major form of IBD, and asthma. A greater number of iNKT cells were seen specifically in the intestinal linings and lungs of mice that were raised in germ-free conditions (GF mice) compared to mice that were raised in an environment of "friendly" bacteria, SPF (specific pathogen-free) mice. Also, the high number of iNKT cells was observed to remain constant for life. All of the study mice were given a chemical substance that mimics UC by inducing inflammatory symptoms. The GF mice responded with far greater sensitivity to UC induction, suffering severe weight loss and higher death rates compared to the SPF mice. To see if inflammation could be prevented in adult GF mice, the adult mice were introduced to "friendly" bacteria before UC induction, but this had no effect on disease development. However, when pregnant mice were exposed to "friendly" bacteria shortly before delivery, the offspring were protected against UC induction, confirming that protection against UC is a time-sensitive phenomenon that is acquired in the presence of "friendly" bacteria early in life.

This type of protection was also demonstrated in a mouse model of asthma. CXCL16 is a pro-inflammatory protein that regulates iNKT cells. In their search for a mechanism underlying susceptibility to UC and asthma, the scientists analyzed the concentration of CXCL16 in samples of mouse blood. The analysis revealed that concentrations of CXCL16, as well as iNKT cells, were significantly elevated in GF mice compared with SPF mice, and that iNKT cells were reduced in the intestines and lungs of mice when CXCL16 interaction with iNKT cells were blocked by antibody interference. Scientists have shown with this study that very early exposure to "friendly" bacteria is necessary to protect against inflammatory responses associated with UC and asthma in mice, and that this protection is enduring. Also, the mechanism for disease sensitivity is dependent on CXCL16 stimulation of iNKT cell inflammatory responses. Although this study was conducted in mice, the mouse system explored and its human counterpart are very similar, and it is expected that these findings will be relevant to developing new treatments for UC and asthma in humans.

Olszak T, An D, Zeissig S, et al. Microbial exposure during early life has persistent effects on natural killer T cell function. Science 336:489-493, 2012.

Early Antibiotic Exposure Changes Gut Microbes and Fat Mass in Mice: A group of scientists has found that even low-dose antibiotic exposure in young mice, similar to that given to farm animals raised in the United States, can dramatically alter the types of microbes present in the gut, as well as specifically increase fat mass. Antibiotic use is high in the United States, for both therapeutic purposes in humans and at subtherapeutic doses to boost growth in farm animals. However, there is some concern about the long-term effects of antibiotic use, not only because of the development of antibiotic-resistant bacteria (so-called "super bugs"), but also for other health effects of such exposures. Antibiotics are known to alter the delicate balance of microbes in the human gut, which is interconnected with energy balance and susceptibility to obesity. Scientists set out to explore how subtherapeutic antibiotic exposure in young animals might alter the gut microbial community, metabolism, and fat mass. They used as their experimental model young mice that had just been weaned. They gave the mice a 7-week course of antibiotics in their drinking water that was equivalent to doses used in the agricultural industry and compared them to mice given no antibiotics. While the mice exposed to antibiotics, as a group, did not differ in overall weight or growth during this period from mice without the exposure, X-ray scans revealed heftier fat mass in the antibiotic-treated mice. Mice given the antibiotics also had elevated levels of a hormone synthesized in the gut that stimulates fat cells. A microbial census taken by analyzing DNA present in the mouse stools and intestinal samples showed that, although antibiotic treatment did not affect the total number of microbes present, it altered the proportions of specific bacterial types. For example, antibiotic treatment was associated with elevations in bacteria called *Firmicutes*, which had been found to be elevated in another mouse model that is genetically prone to obesity. In the colon, major increases were observed in short-chain fatty acids, a product of complex carbohydrate metabolism by bacteria that can be used by colon cells for energy or can be absorbed into the circulation and stimulate fat tissue formation.

The liver also showed altered levels of gene activity associated with fat metabolism. Although the mice given antibiotics consumed the same amount of food as their non-treated counterparts, their fecal pellets showed less caloric content wasted, suggesting that their altered microbial community was more capable of extracting calories from the diet. This study provides a mechanism for the increased mass of farm animals given low-dose antibiotics. It also suggests that even low-level antibiotic exposure in young animals, and potentially humans, may come with an increased risk of obesity by increasing the numbers and activity of gut microbes that are more efficient at harvesting energy from the diet.

Cho I, Yamanishi S, Cox L, et al. Antibiotics in early life alter the murine colonic microbiome and adiposity. Nature 488: 621-626, 2012.

Gut Microbes Transmit Susceptibility to Nonalcoholic Fatty Liver Disease Progression: Researchers have shown how changes in the bacteria in the gut play a surprising role in the progression of nonalcoholic fatty liver disease (NAFLD). NAFLD is the liver's manifestation of metabolic syndrome, a group of risk factors that together increase the risk for cardiovascular disease and type 2 diabetes. In some people, the disease takes a relatively benign form, with excess fat accumulation in the liver, while in others, the disease progresses such that fat accumulation is accompanied by inflammation, liver damage, and scarring, called nonalcoholic steatohepatitis, or NASH. The cause of progression to the more severe form of the disease is still not well understood. However, in light of the direct connection between the intestine and the liver through the portal vein, scientists wondered if gut microbes, and the human host's microbial response system, might play a role in progression of this disease. Starting with the human immune system, the research team focused on inflammasomes—complexes of immune proteins involved in sensing microbes and triggering responses to those that are harmful. Mice that were genetically modified to lack some inflammasome components developed NASH when fed a special diet, showing that these human immune factors play a key role in putting the brakes on progression of this disease. But, even more interesting was that when these mice with genetic defects in microbe-sensing and severe NASH were housed together with "normal" mice without any genetic alterations or liver disease, the normal mice also developed the severe form of liver disease. The scientists concluded that a unique mix of intestinal bacteria in these mice lacking proper microbe-response machinery may have been transmitted to the normal mice, carrying the susceptibility to develop severe liver disease with it. They next sequenced genes characteristic of different types of bacteria from the intestines of the mice to identify the species present. They found a few species in particular that were present in unusually high numbers compared to intestinal bacteria in the normal mouse intestine. They also showed how these intestinal bacteria might bring about NASH by releasing their products into the blood and activating immune factors in the liver that lead to inflammation and damage. In addition, they examined another mouse model, this one genetically modified to be obese; these animals also "caught" the susceptibility to develop severe NASH from the inflammasome-deficient mice. These animals became even more obese and showed signs of increased insulin resistance. These studies have greatly expanded the understanding of how NAFLD's progression to NASH is related to intestinal microbes. More research is needed to tease out contributions by particular microbial species. However, this work may provide a basis for future antibiotic/probiotic therapies for individuals susceptible to developing NASH.

Henao-Mejia J, Elinav E, Jin C, et al. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. Nature 482: 179-185, 2012.

GENETICS OF INFLAMMATORY BOWEL DISEASE

Rare Genetic Variants Identified for Inflammatory Bowel Disease: New technologies have enabled scientific researchers to uncover important rare genetic mutations associated with inflammatory bowel disease (IBD). Crohn's disease (CD) and ulcerative colitis (UC), the two major forms of IBD, are complex diseases attributed to errant immune responses caused by a

combination of genetic variants and environmental factors. Some variants are unique to either CD or UC, while others are shared. Using genome-wide association studies (GWAS) technology, as of November 2012, scientists had identified 163 genomic regions with variants that contribute to IBD. The variants that are known, however, account for only a small portion of IBD disease risk, and scientists have not yet identified the disease-associated gene within many of the implicated genomic regions. Scientists from several IBD research consortia and teams around the world have now used pooled "next-generation" sequencing technologies to examine 56 genes located in CD-associated genomic regions that had been identified by GWAS. Using pooled DNA samples from 350 CD patients and 350 healthy volunteers collected by the NIDDK IBD Genetics Consortium, IBD risk-related gene regions were sequenced using high-throughput sequencing technology. The scientists developed computer software to analyze the IBD sequencing data, which led to discovery of important rare variants. They further examined these variants in over 16,000 people with CD and 12,000 with UC, in comparison with more than 17,000 healthy individuals. A highly significant variant that confers IBD protection was identified in the *CARD9* gene. Two additional rare protective variants were identified in the *IL23R* gene and one in the *CUL2* gene. Five CD risk variants were identified in the *NOD2* gene. Two of the *NOD2* variants are more common in people of Ashkenazi Jewish ancestry—a population that is at high risk for CD—and one of the two variants is found only in the Ashkenazi population. In addition, associations in other gene coding regions were identified. This research has led to the discovery of multiple genetic variants conferring disease risk or protection in a number of IBD-associated genes previously identified by GWAS. These variants will provide valuable insights into the mechanisms by which these genes influence susceptibility to IBD. In addition, the identification of the *CARD9* variant, which offers disease protection, has the potential to lead to new therapeutic models for IBD prevention and treatment.

Rivas MA, Beaudoin M, Gardet A, et al. Deep resequencing of GWAS loci identifies independent rare variants associated with inflammatory bowel disease. Nat Genet 43:1066-1073, 2011.

New Crohn's Disease Gene Variants Identified in Ashkenazi Jewish Population: Scientists have discovered new genetic variants associated with Crohn's disease (CD) while investigating the Ashkenazi Jewish (AJ) population's high risk for this disease. The AJ, who are of Eastern and Central European descent, have a two- to four-fold greater risk of developing CD than Europeans of non-Jewish descent. Since the most frequent CD susceptibility variants discovered thus far in the AJ population are similar to those found in non-Jewish populations, those variants do not account for the higher risk for people of AJ heritage. For this study, scientists used new methods and technologies to identify unique genetic variations in this high-risk population. To mine the genetic information of this at-risk group, scientists gathered the largest number of AJ CD cases that has been assembled to date. This was accomplished by combining samples from several previous genome-wide association studies (GWAS), including the NIDDK IBD Genetics Consortium. To confirm the ancestry of the study participants, the researchers used genetic analyses to distinguish AJ ancestry from other populations, including non-Jewish Europeans and other Jewish populations. With additional analysis, the scientists were able to further distinguish AJ participants who had different degrees of AJ heritage—100 percent, 75 percent, or 50 percent, for both separate and combined analyses. The researchers then compared genomic data from AJ individuals with and without CD to identify genetic variants associated with this disease, and also compared genomic data from AJ study participants to data from non-Jewish individuals of European descent. These studies resulted in the discovery of five novel regions associated with CD in the AJ population, and the confirmation of several previously identified variants. The new and confirmed variants account for 11.2 percent of the total genetic variance for CD risk in the AJ population. This research demonstrates the complementary value of genetic studies in the Ashkenazi Jewish and other at-risk populations. The newly defined CD variant regions provide the basis for further studies of the biological pathways responsible for CD and may result in novel treatments.

Kenny EE, Pe'er I, Karban A, et al. A genome-wide scan of Ashkenazi Jewish Crohn's disease suggests novel susceptibility loci. PLoS Genet 8: e1002559, 2012.

MECHANISMS OF LIVER AND BILIARY DISEASES

Hepatitis B Virus Infection Control by Early Immune Cell Sensing: Scientists have shown how an immune cell called the natural killer T cell mounts an early defense against hepatitis B virus infection by sensing modified fat molecules produced by infected liver cells. While most healthy adults clear the hepatitis B virus (HBV) on their own, some groups such as children are more vulnerable to chronic infection. The immune system has two main types of defenses against pathogens like HBV: innate immunity, which includes early cellular responders that attack invaders, and adaptive immunity, which involves activation of other cell types and the production over several days of specific antibodies against the invaders. Natural killer T (NKT) cells occupy a unique niche by responding early to pathogens as part of innate immunity, but then activating other cells that participate in both types of immune defense. Scientists set off to investigate what type of role these NKT cells might play in early defense against infection by HBV, with experiments in mice and in isolated liver cells. Because HBV does not naturally infect mouse liver cells, the scientists infected mice using a modified vector to deliver the HBV genome. As soon as 1 day after infection, NKT cells became activated in the liver, but not elsewhere. Using mice that were genetically modified to lack NKT cells and then infected with HBV, the researchers showed that activation of other immune cells typically stimulated by NKT cells was diminished, resulting in higher viral levels, fewer antibodies, and more hepatitis. In cell culture, they showed that mouse and human liver cells infected with HBV were necessary for the NKT cells to become active. When the liver cells became infected with HBV, they produced unique fat molecules (lipids) that signaled their infection to the NKT cells, which responded by producing chemicals that mount a full immune response and play a role in clearing the infection. These findings suggest that NKT cells contribute to early immunity against and clearance of HBV by sounding an alarm that alerts the immune system to the viral intruder. This research points to potential approaches to preventing and treating chronic HBV infection through targeting these cells.

Zeissig S, Murata K, Sweet L, et al. Hepatitis B virus-induced lipid alterations contribute to natural killer T cell-dependent protective immunity. Nat Med 18: 1060-1068, 2012.

Transparent Fish Illuminates Formation of Ductal Network: Scientists have employed a see-through fish for their research into the molecular mechanisms underlying formation and loss of the network of ducts connecting the liver, gallbladder, pancreas, and intestine. A system of ducts carries bile from the liver to the intestine to aid in fat digestion, with some bile stored in the gallbladder. Additionally, this ductal network carries digestive enzymes from the pancreas to the intestine, where they aid in digestion and absorption of nutrients. Some congenital conditions in humans result from improper formation or loss of these ductal networks. Two recent studies of these ductal networks utilized the zebrafish—a common animal model for research due to its ease of breeding and its transparency, which allows organ systems to be visualized directly.

In one study, scientists used a genetic screen and a fluorescently labeled marker to identify factors that regulate liver development in zebrafish larvae. They found that a gene, called *snapc4*, was important for liver development. The *snapc4* gene codes for a protein, similarly named snapc4, which regulates other genes with respect to whether they are on (expressed) or off. Livers of zebrafish with mutated (defective) *snapc4* were much smaller than normal. Further investigation of these mutants showed blocked transport of lipids consumed by the fish from the intestine to the gallbladder, indicating impaired functioning of the biliary duct network. By closely monitoring development of the larvae after fertilization, they observed that the biliary duct network initially formed, but the cells then died by a process called apoptosis and disappeared later in development. Additionally, the team found that the defective snapc4 protein was impaired in

its ability to bind with another protein called snapc2 in a larger functional complex, suggesting that both proteins are important for maintaining the bile duct network. The *snapc4* mutant zebrafish larvae showed features similar to the human disorders known as biliary atresia and vanishing bile duct syndrome, which are marked by biliary duct destruction from the disappearance of differentiated biliary epithelial cells through apoptosis. Future research could help determine if snapc4 and snapc2 play a role in this disorder.

Another research team used the zebrafish to investigate development of the ducts connecting the liver, pancreas, and intestine. They zeroed in on a gene called *sox9b*, which encodes a protein, sox9b, involved in regulating gene expression (although different from the factor identified in the other zebrafish study). *Sox9b* itself is expressed specifically in the ductal system. While researchers had previously identified the mouse version of *sox9b*, it had been difficult to study because even one copy of a mutated form of this gene is lethal for mice. Zebrafish, however, survive to adulthood with two genes encoding non-functional proteins, enabling studies of the effect of this gene on ductal development. Zebrafish with the non-functional form of *sox9b* exhibited impairment of the ductal system early in development, with blocked bile flow, as well as cells that should become liver and pancreatic ductal cells mistakenly taking on characteristics of the wrong cell types. Using fluorescently labeled markers for specific cell types, the scientists saw that the fish with this non-functional sox9b protein displayed a malformed network of ducts connecting the liver, pancreas, and intestine. Additional experiments showed that the sox9b protein is also important in maintaining ductal cell signaling through another pathway involved in early organ development through a protein called Notch. These findings shed new light on development of the ductal system and possible candidate genes (*e.g.*, the human *SOX9* gene) underlying human conditions with similar features such as biliary atresia or Alagille syndrome.

Schaub M, Nussbaum J, Verkade H, Ober EA, Stainier DY, and Sakaguchi TF. Mutation of zebrafish Snapc4 is associated with loss of the intrahepatic biliary network. Dev Biol 363: 128-137, 2012.

Delous M, Yin C, Shin D, et al. sox9b is a key regulator of pancreaticobiliary ductal system development. PLoS Genet 8: e1002754, 2012.

LIVING DONOR LIVER TRANSPLANTATION RESEARCH

Understanding Risk of Complications in Living Donors Undergoing Liver Transplantation:

A clinical study conducted at multiple sites across the United States has extensively described donor risk of long-term complications from living donor liver transplantation. Liver transplantation is the only option for those with end-stage liver disease, but the supply of organs available from deceased donors is severely limited relative to demand. Living donor liver transplantation can alleviate this problem, but potential risks to the donor must be investigated thoroughly prior to widespread use. For this purpose, the NIDDK's Adult-to-Adult Living Donor Liver Transplantation cohort study undertook an assessment of the incidence, severity, and natural history of donor complications from living liver donation. At 9 transplant centers throughout the United States, the investigators studied over 700 living liver donors over a 12-year period, the longest ever for a study of complications in these donors. They found similar rates of complications to previous, shorter-term studies, with 40 percent of donors having one or more complications and 19 percent experiencing multiple complications. The donors' estimated chances of disability, liver failure, or death after the procedure were 1 percent. Within the first few weeks after the transplant procedure, the most common complications were infections, excess fluid around the lungs, bile leaks, nerve damage, and bowel obstruction. Most of these complications resolved within 3 months after the procedure and, overall, 95 percent of complications were resolved within 1 year. Longer-term complications in the following months or even 5 to 6 years later included hernia, bowel obstruction, and psychological complications. Factors predicting a greater chance of complications included the donor's need for blood transfusion and low blood pressure during the procedure, features that are characteristic of more prolonged and complicated

surgery. Other factors that predicted specific serious complications included higher body weight, older age, and male gender. Surprisingly, among the nine transplant centers participating in the study, all of which had past experience in performing liver transplants, the extent of the center's experience in performing the procedure did not significantly predict donor complications. Further research will be needed to assess the long-term risk to donors of living donor liver transplantation as this procedure continues to evolve. These findings can be used to focus efforts on reducing the rate of complications and are invaluable in aiding the decision-making process of individuals who are considering becoming living liver donors and their loved ones.

Abecassis MM, Fisher RA, Olthoff KM, et al. Complications of living donor hepatic lobectomy—a comprehensive report. Am J Transplant 12: 1208-1217, 2012.

UNDERSTANDING INTESTINAL IRON TRANSPORT

Discovery of a New Protein Involved in Intestinal Iron Transport: Scientists have discovered a new protein in rodents involved in intestinal iron transport, which may complement actions of other proteins required for facilitating absorption of this important nutrient. Iron is absorbed from food by cells of the small intestine and used for such essential functions as red blood cell production. In cases where insufficient iron is absorbed, anemia can result. Iron transport out of cells lining the intestine and into the circulation was thought to require an enzyme—hephaestin—that spans the cells' membranes. This enzyme oxidizes iron into a form that can be transported out of the intestinal cells. But, when scientists created a mouse with a mutated, inactive form of hephaestin, the animals were only mildly iron-deficient, suggesting another protein was compensating for the lost hephaestin. Scientists have since undertaken a search for a new enzyme with iron-oxidizing capabilities similar to those of hephaestin. They also considered the possibility of compensation by another enzyme known to oxidize iron, called ceruloplasmin. For this research, they used rat and mouse models with genetic mutations in these iron-oxidizing enzymes, as well as models with mild, diet-induced deficiencies in either iron or copper, because activity of these enzymes is known to increase when dietary iron levels are low, and both enzymes also happen to require copper in order to perform their functions on iron. Samples of intestinal cells taken from iron-deficient rats with elevated iron-oxidizing enzymes were broken open and processed to separate the cells' internal contents (the cytosol) from those in the cell membrane. Surprisingly, iron-oxidizing activity was detected in the cytosol, as well as in the cell membrane. Experiments with the rodents that had genetic mutations showed that iron was still oxidized, confirming the presence of a previously unknown iron-oxidizing enzyme in the cytosol of intestinal cells. This discovery of a new iron-oxidizing enzyme inside rodent intestinal cells, which may work in concert with the membrane-bound hephaestin to enable iron transport, deepens understanding of mammalian iron transport processes and related conditions.

Ranganathan PN, Lu Y, Fuqua BK, and Collins JF. Discovery of a cytosolic/soluble ferroxidase in rodent enterocytes. Proc Natl Acad Sci USA 109: 3564-3569, 2012.

NIDDK Grantee Dr. Thomas E. Starzl Wins 2012 Lasker Award

Dr. Thomas E. Starzl (left), winner of a 2012 Lasker award for his pioneering efforts in organ transplantation, is congratulated by NIDDK Director Dr. Griffin P. Rodgers (right) at the Lasker Awards ceremony on September 21, 2012. ***Photo credit: Ellen Jaffe***

Dr. Thomas E. Starzl, Distinguished Service Professor of Surgery at the University of Pittsburgh School of Medicine and a long-time NIDDK grantee, received the 2012 Lasker-DeBakey Clinical Medical Research Award—shared with Dr. Roy Calne, University of Cambridge emeritus—for his work developing liver transplantation, an intervention that has restored normal life to thousands of people with end-stage liver disease.

Lasker awards are given for major advances in the understanding, diagnosis, treatment, cure, and prevention of human disease. Dr. Starzl performed the first human liver transplant. In addition to being a long-time NIDDK grantee, he is also a former NIDDK Method to Extend Research in Time (MERIT) awardee and has served on the NIDDK Digestive Diseases Advisory Board. He also earned a 2004 National Medal of Science.

"Dr. Starzl is a pioneer in the world of transplantation, and his work has saved thousands of lives," said NIDDK Director Dr. Griffin P. Rodgers. "This award is a most fitting recognition of his many years of unwavering commitment to teaching, research, and clinical practice."

Receiving the award on September 21, 2012, Dr. Starzl said, "Transplantation services are not provided by single individuals. The team is what counts, and it is on behalf of my research and clinical teams—first in Denver and then in Pittsburgh—that I accept this prize. And by the way, the prize could have gone to one of those courageous kidney, liver, or heart recipients who faced the great unknown in the early years and chose to run the uncharted gauntlet of transplantation instead of giving up. Win or lose, these were the heroes."

LiverTox: A New Online Resource for Information on Drug-Induced Liver Injury

The NIDDK's Liver Disease Research Branch, in collaboration with the National Library of Medicine's Division of Specialized Information Services, has developed an online resource for information on drug-induced liver injury resulting from prescription and over-the-counter drugs as well as from complementary and alternative medicines such as herbals and dietary supplements. Called "LiverTox," this web-based resource provides up-to-date, accurate, and easily accessible information on the diagnosis, cause, frequency, patterns, and management of liver injury attributable to these agents.

Liver injury from medications, herbals, or dietary supplements has emerged as an increasingly important health problem in the United States. Although most cases of liver injury are mild and resolve quickly, some individuals develop liver injury so severe that it can lead to acute liver failure and, ultimately, transplantation or death. In the United States, liver injury due to drugs is the leading cause of acute liver failure, occurring with increasing frequency in recent years based on findings from the NIDDK's Acute Liver Failure Study Group.

One of the challenges in treating this form of liver injury is the accurate and timely identification of the drug(s) causing the injury so that steps can be taken to limit the damage. Different drugs or supplements can cause disparate patterns of liver injury that can sometimes mimic other forms of liver disease, making it difficult for physicians to recognize unless they first rule out all other potential causes of liver injury.

The creators of LiverTox set out to remedy this by providing a comprehensive source of information to aid health care providers and patients in diagnosing, and researchers in studying, liver injury due to specific drugs, herbals, or dietary supplements. The web-site serves as a centralized "one stop shop" for information relating to the prevention and control of drug-induced liver injury from multiple agents, as well as guidance on the diagnosis and management of this important cause of liver disease.

LiverTox has three major components: 1) an introduction and overview of drug-induced liver injury, 2) a series of individual drug records or monographs on specific medications, herbals, and dietary supplements describing their liver toxicity with specific case histories and a complete set of references, and 3) a case submission registry that allows users to report a case directly to LiverTox and which then can be forwarded to the U.S. Food and Drug Administration's Adverse Event Reporting System (MedWatch). The LiverTox database currently includes information on approximately 700 drugs or supplements available in the United States, and the current plan is to add 300 more over the next few years. Case reports of liver toxicity were collected from several sources, including the published scientific literature, the database of the NIDDK's Drug-Induced Liver Injury Network, and cases seen at the NIH Clinical Center.

LiverTox's creators envision it as a "living textbook" with ongoing updates and improvements. They will continue to draw upon the collective wisdom of the wider scientific and health care community by welcoming comments and information on all known drug- and supplement-related forms of liver injury from web-site users, in hopes of reducing these forms of liver injury in the future.

The LiverTox web-site is accessible at:
http://livertox.nih.gov/

Workshop Charts Progress and Promise of Pancreatitis Research

In June 2012, the NIDDK convened a 2-day research workshop at the NIH campus in Bethesda, Maryland, on "Advances in Acute and Chronic Pancreatitis: From Development to Inflammation and Repair." The workshop provided a state-of-the-art update on a wide range of research efforts addressing acute and chronic pancreatitis and charted a course for advancing future research in this area.

Pancreatitis is a disease marked by inflammation of the pancreas; the inflammation results from aberrant activation of digestive enzymes. Normally, the pancreas releases digestive enzymes that subsequently become activated within the intestine to aid the digestion of food. In pancreatitis, the enzymes become activated while still within the pancreas, causing tissue damage and pain. Pancreatitis can be acute, with inflammation resolving within a few days, or chronic, involving long-term inflammation and tissue damage. A variety of factors can contribute to the development of pancreatitis, including genetics, gallstones, heavy alcohol use, and other causes. Currently, there are no cures or preventive therapies for pancreatitis. The NIDDK actively supports research on pancreatitis, including clinical trials such as the North American Pancreatic Study 2 and Study of Nutrition in Acute Pancreatitis.

Major drivers for organizing this timely workshop were current gaps and opportunities in pancreatitis research. The workshop was co-organized by NIDDK staff; two members of the NIDDK Advisory Council who are active in this area, Dr. Anil Rustgi, Chief, Division of Gastroenterology, University of Pennsylvania, School of Medicine, and Ms. Jane Holt, co-founder of the National Pancreas Foundation; as well as others in the external pancreatitis research community.

The objectives guiding this workshop were:

- To enhance understanding of pancreatic developmental and stem cell biology, acute and chronic forms of pancreatitis, genetics of pancreatitis, the link between the inflammatory pancreatic microenvironment and neoplasia, and pancreatic neurobiology;
- To identify new strategies in diagnosis, imaging, and therapy of pancreatitis;
- To foster collaborative and innovative interdisciplinary translational research; and
- To coalesce the meeting findings into a publication as a roadmap for future NIH-based initiatives.

Topics discussed over the 2 days ranged from basic research on pancreatic development and regeneration to mouse models of pancreatitis and new treatment strategies based on genetic susceptibility and environmental factors. Some participants described results from efforts supported by the NIDDK, such as studies conducted through the Beta Cell Biology Consortium and North American Pancreatitis Study 2. Presenters came from around the globe to share their findings, representing research institutions from the United States, England, Canada, Germany, and Spain. Participants also shared research resources, such as the "Pancreapedia" web-site, which is supported in part by the NIDDK (*www.pancreapedia.org*).

This productive workshop continues to bear fruit by guiding investigators toward promising future research directions in this area. Consistent with one of its objectives, a report summarizing the meeting was published in January 2013 in the journal *Gastroenterology* (144: e1-e4, 2013), in order to share knowledge and recommendations with the larger research community focused on advancing understanding and improving care for those with acute and chronic pancreatitis.

STORY OF DISCOVERY

Alpha-1 Antitrypsin Deficiency—From Genes to Therapies

NIH-funded research over the past decades has helped to decipher the genetic underpinnings and clinical manifestations of alpha-1 antitrypsin (AAT) deficiency, an inherited disorder associated with liver disease, as well as disease in other organs such the lungs. Although the disease is caused by a single abnormal gene, its manifestations vary greatly depending upon the specific mutation a person has, whether a person inherits copies of the abnormal gene from one or both parents, and also on other genetic and environmental factors. With knowledge gained from research has come an expanded understanding of AAT deficiency, the differences among its various forms, and development of new therapeutics based on this scientific foundation.

The AAT enzyme is synthesized in the liver and secreted into the bloodstream, where it is transported throughout the body to help protect against tissue damage. It plays a particularly important role in the lungs, where it prevents the breakdown of proteins in connective tissue that help the lungs remain flexible.

AAT deficiency is a genetic disorder in which the gene encoding AAT is mutated, resulting in lower levels of this protein in the blood and diminished AAT activity in the lungs. AAT deficiency is a major contributor to chronic obstructive pulmonary disease and emphysema due to lung damage. The lower levels of AAT in the lungs are a consequence of the retention of malformed AAT protein in the liver, where its accumulation can cause tissue damage. AAT deficiency is the most common genetic cause of liver disease in children and an uncommon but important cause of liver disease in adults, sometimes leading to chronic liver disease and liver cancer. Patients with AAT deficiency thus face potential health risks on two fronts: lung disease, due to insufficient circulating AAT, and liver disease, due to accumulation of AAT in this organ. Research has greatly increased scientists' understanding of the molecular processes involved in AAT deficiency, and recent studies have highlighted a promising new approach to addressing the sometimes life-threatening consequences of this serious condition.

Molecular and Cellular Features of AAT Deficiency

Since the mid-1960s, scientists have identified over 120 variants of the AAT gene. These variants are grouped into three categories based on the level of AAT they release into the bloodstream—normal, deficient, or virtually undetectable. About 100,000 Americans have the most severe form of AAT deficiency. In these patients, a significant fraction of the mutant AAT protein does not complete its journey from the interior of a liver cell, where it is assembled, to the cell surface, where it would normally be released into the bloodstream. Rather, the mutant protein forms polymers—chains of AAT molecules—which aggregate within liver cells at a site that acts as a checkpoint for quality control of proteins. The retention of polymerized AAT within the liver has two adverse consequences: first, the accumulation of AAT polymers results in tissue damage, including inflammation and fibrosis, in this vital organ; second, it results in lower levels of AAT in the bloodstream, which means that this protein cannot perform its important protective role of keeping in check the tissue-degrading enzymes in distal organs, especially the lungs.

Clinical Characterization of AAT Deficiency

The importance of AAT was originally recognized in studies of blood proteins in 1963, when scientists

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found that some patients with emphysema lacked sufficient amounts of AAT in their blood. In 1969, other researchers observed that patients with a particular variant of the AAT gene had a high frequency of liver disease, including neonatal jaundice and cirrhosis. In addition, the patients had high concentrations of the variant AAT in their liver cells. These and other observations about the disease enabled fundamental research to begin uncovering underlying mechanisms—a prelude to the development of treatments.

Scientists supported by the NIH have focused vigorous research efforts on liver damage arising from AAT deficiency. Because not all people with variant AAT develop liver disease, researchers have searched for factors that might predispose some patients to be susceptible to or protected from this liver damage. To this end, in 1994, researchers grew skin cells from AAT-deficient individuals who had never suffered from liver disease and who therefore might be "protected." Similar cultures were made with cells from AAT-deficient individuals who had severe liver disease and were therefore considered "susceptible." While cells from both cultures accumulated the variant AAT protein, only the "susceptible" cells exhibited a delay in degrading this abnormal protein, suggesting that some AAT-deficient patients have alterations in the degradation pathway for the protein in their liver cells—alterations that may predispose them to developing liver disease.

Possible Treatments for AAT Deficiency

In the 1980s, scientists demonstrated that increasing a patient's levels of functional AAT by administering the normal form of the protein was feasible and beneficial. AAT-deficient patients achieved an increase in their blood levels of the normal protein following the intravenous transfusion of purified AAT from the blood of healthy individuals. Further research led to the FDA approval of a purified form of the enzyme for the treatment of AAT-related lung disease in 1987. Related therapies on the horizon are intravenous AAT augmentation products, inhalation delivery systems, and synthetic augmentation therapies. However, while these therapies for AAT-related lung disease are promising, they do not address the other manifestation of AAT deficiency: liver disease.

In the late 1980s, a study of mice engineered to produce a mutant human form of AAT secreted sufficient AAT into their bloodstream to protect them from lung disease but still suffered from a build-up of AAT in their liver cells and showed signs of liver damage. In 1989, additional research supported by the NIH substantiated that the aggregation of AAT protein within liver cells caused disease. Subsequent studies showed that patients with AAT deficiency sustain liver damage due to inflammatory immunological responses to the aggregated protein, and that the immunosuppressive drug cyclosporin A could help prevent AAT liver damage—a proof-of-principle for immune mechanism-based therapeutic approaches to AAT deficiency. All of these studies point to the accumulation of AAT as a key factor in the development of liver disease in AAT deficiency.

Building on these findings, more recent studies have focused on strategies to limit the accumulation of malformed protein in the liver to address the manifestations of AAT deficiency in this organ. One approach has targeted "autophagy"—literally, "self-eating"— the process by which a cell breaks down and recycles its components. In 2010, basic research studies demonstrated that, in liver cells, increased autophagy affords limited protection from damage caused by AAT aggregates. Treatment with the drug carbamazepine markedly reduced the amount

of accumulated protein in cultured cells by boosting autophagy activity, and this drug also reduced liver fibrosis in a mouse model of AAT deficiency. Notably, carbamazepine has been used as an anticonvulsant and mood stabilizer in humans for over 40 years and its safety and tolerability are well known. Using a system to screen large numbers of drugs, investigators are searching for agents that improve autophagy and reduce the AAT aggregates in cells. Targeting the autophagy pathway may be a promising strategy for future therapeutic approaches.

Looking Forward

Despite recent progress toward new therapies, the only effective treatment for liver failure due to AAT deficiency is liver transplantation, for which donor organs are severely limited. Therefore, there remains a need for alternative therapies that can treat or prevent the serious liver disease that often accompanies AAT deficiency. By combining basic research on cellular processes underlying disease with knowledge of existing therapeutics that target these processes, researchers are striving to identify promising treatments that may work for multiple diseases. While autophagy-enhancing drugs are a promising potential treatment for liver disease associated with AAT deficiency, further studies will be needed to test the benefits and risks of this treatment in pediatric and adult patients with this serious form of liver disease.

The NIDDK supports a broad range of research related to liver disease from AAT deficiency in children and adults. One example is the Childhood Liver Disease Research and Education Network (ChiLDREN), which was created by joining the Cholestatic Liver Disease Consortium and the Biliary Atresia Research Consortium. ChiLDREN includes studies of AAT deficiency and is funded by the NIDDK with substantial support by the Alpha-1 Foundation. Through research on its collection of clinical data and biospecimens, this Network is poised to gain a better understanding of how AAT deficiency leads to liver disease, as well as to contribute to the development of new treatments for this condition.

PATIENT PROFILE

Marcia Chichester

Her "Brother's" Keeper—Two Friends Bound Together by Living Donor Liver Transplantation

Marcia Chichester (right) with Jason Donley (left)

In 2011, Marcia Chichester received her registered nursing (RN) degree after working 21 years as a licensed practical nurse. To mark this important achievement, like other RNs before her, she participated in a pinning ceremony where someone special to her attached her RN pin. She chose her good friend and former co-worker Jason Donley to perform this honor. But, as Jason walked across the stage and attached Marcia's pin, what outside observers couldn't see was the deeper bond these two friends share—he was alive and well that day because of her selfless gift 6 years before.

Faith and Friendship

In the early 2000s, Marcia and Jason were both nurses at a hospital in Charlotte, Michigan. Marcia, then a single mom with two teenage sons and working two nursing jobs, offered to spend her day off to drive Jason to a doctor's appointment 3½ hours away to see a specialist who treated his primary sclerosing cholangitis or "PSC." PSC is a form of liver disease marked by inflammation and scarring of the bile ducts leading from the liver to the intestine, which causes bile to back up into the liver and damage the organ.

As a teenager, Jason was diagnosed with ulcerative colitis, and then was diagnosed with PSC after college. Both PSC and ulcerative colitis are forms of autoimmune disease that often occur together and run in families. Over time, PSC causes a yellowing of the skin called jaundice, pain along the right ribs, nausea, and weight loss. "I started glowing in the dark," he recalls. "It was hardest watching my family deal with it. My mom would lift my shirt up and put her arm next to my tummy to see how yellow I was. And if I was really yellow, she'd just go off and cry."

But the worst symptom for Jason was the intense and insatiable itching caused by the liver disease. "The itching will drive you insane," he recalls. "It's an itch you just really can't quench." The itching was intensified by sweating, so he was careful to maintain cool temperatures at home, take regular ice water baths, and minimize physical activity. Despite the challenges of living with PSC, Jason threw himself into his work, taking on extra shifts to distract himself from the itching. But, he knew that it was only a matter of time until the disease progressed to a point where he would need a liver transplant.

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After that long trip together, Marcia and Jason became good friends, and, along with another co-worker, formed a bible study group. Marcia also continued to accompany him to all his out-of-town doctor's appointments. On one appointment in February 2005, Jason's doctor informed them that he would need a transplant soon and should consider living donor liver transplantation. A few years before, he and Marcia had learned about the procedure at a liver transplant orientation meeting at Northwestern University in Chicago, Illinois. At that meeting, they also heard about a clinical study on this procedure called the Adult-to-Adult Living Donor Liver Transplantation Cohort Study (A2ALL), and they both remember being enthusiastic about the study. (See box at end of profile for more information on A2ALL.)

Jason considered a living donor transplant, but his immediate family members were not eligible to donate due to his siblings' own autoimmune diseases and the age of his parents. He also began the process of getting on the waiting list for a deceased donor organ, though his chances of receiving one in time were slim due to the high demand and the rules of the system used to allocate these organs. As Marcia watched Jason grow sicker, she read online about a woman who donated part of her liver to a non-relative. Marcia resolved to be Jason's donor, if possible, and discussed it with her family.

"Well, he was sick and going to die. And I told my boys, 'I might be a match'—they were 18 and almost 15 at the time," remembers Marcia. "They both said 'You've got to do it, Mom.' We're a family with a really strong faith, and we knew that if it was meant to be, it would work and everything would be fine." She informed the rest of her family, then visited Jason to tell him that she wanted to be his living donor. He was concerned about the impact of such a decision on his friend and her family, particularly her teenage sons, and discussed it with them to ensure all were supportive of the decision.

Marcia resolved to be Jason's donor, and discussed it with her family. "He was sick and going to die. And I told my boys, 'I might be a match'," she remembers. "They both said 'You've got to do it, Mom.'"

Jason chose to have the transplant performed at Northwestern, where Marcia underwent a series of tests to evaluate whether she could donate to him. They were a match for blood type, but a liver imaging test found some fat in her liver, which can be a marker of fatty liver disease and compromise functioning of the organ in both a potential donor and recipient. That made her ineligible to donate, but she was determined. "I went to my doctor and said 'How do I get rid of this?'," she recalls, referring to the liver fat. Her doctor recommended a weight loss program, as she was overweight at the time. She immediately went on a strict diet and rigorous exercise regimen in an effort to lose weight and reduce the fat in her liver.

Meanwhile, Jason was running out of time. Weeks later, he became very ill, and when they went to Northwestern for his appointment, Marcia asked to be tested again. The nurse warned her that if this second test was also positive for liver fat, she would not be allowed to be tested again. Again, Marcia was undeterred and insisted on a retest. Surprisingly, the second test found no fat in the liver, and she was permitted to complete the rest of the matching process for donating part of her liver to Jason. She met all of the criteria, and they set a date for the transplantation, deciding also to enroll in the A2ALL Study. The good news came just in time—Jason was expected to live only a few months without a liver transplant.

PATIENT PROFILE

Living Donor Liver Transplantation

The liver is an organ with two lobes located in the abdomen beneath the ribs that is required for survival. Every day, it silently multi-tasks—processing nutrients, neutralizing toxins, and performing a number of other vital functions. It also shows a remarkable capacity for partial regeneration, or being able to regrow after injury. But when damage occurs to the point where liver function is severely compromised by disease, as was the case with Jason, the organ cannot regenerate adequately, and a liver transplant is the only option.

Initially, liver transplants were only performed using organs from deceased donors. However, because the need for livers for transplantation is far greater than the number of deceased organs available, alternative approaches were needed. Due to the liver's capacity to regenerate, living donor liver transplantation was a therapeutic possibility, and the first living donor transplant was performed in the late 1980s. Further refinements in living donor liver transplantation have improved the outcomes in both donors and recipients, although complications from the procedure still occur. As with any major surgery, there is a risk of death from living donor liver transplantation, and donor deaths have occurred, even at well-established transplant centers with experience performing the procedure. True to their Hippocratic oath of "first, do no harm," doctors are especially concerned with finding ways to prevent complications and death in the healthy living donor. Research, such as the A2ALL Study that Marcia and Jason decided to participate in, aims to provide insights into the risks and benefits of the procedure.

A Complicated but Rewarding Journey

In July 2005, Marcia and Jason, accompanied by their families and a few close friends, returned to the hospital at Northwestern for the living donor liver transplant procedure. He was now extremely ill—a vivid shade of yellow and gaunt, down to around 160 pounds on his 6-foot frame. The group arrived early and spent a day touring Chicago together. "We decided we were going to have one fun day and then if something happened, we would have that day to remember," recalls Marcia.

At the hospital the following day, both Marcia and Jason were being prepared for the surgery when they were informed that an emergency situation had arisen that required the attention of the same transplant team. The pair graciously agreed to delay their surgery until 5 days later, returning to Michigan in the meantime.

On July 18, 2005, Marcia, Jason, and their families held a prayer session before heading over to the now-familiar hospital at Northwestern. They were taken to separate rooms in the surgical ward and prepared for surgery. Over the next several hours, the transplant team replaced Jason's liver with the right lobe of Marcia's liver.

Within just a few days of the transplant, Jason recovered with remarkable speed as his new liver immediately began performing the vital functions that had been compromised by the PSC for so long. The day after surgery, 95 percent of his jaundice was gone, and he was walking the hospital hallway. For the first time in many years, his skin was no longer yellow, and the itching that had tormented him was gone. By the time of their 3-month check-up, both Marcia's and Jason's livers had regenerated to over 90 percent of their full size.

But the procedure was not without complications. As they recovered from their surgeries, their shared religious faith, along with the support of one another, friends, and family, would see them through some difficult times ahead. A few days after the surgery, Marcia was back in the hospital with a painful bowel obstruction. Now it was Jason's turn to act as his

PATIENT PROFILE

"sister's" keeper, visiting her and vigilantly monitoring her charts and care until she recovered. By 1 month after transplantation, both Marcia and Jason were able to return home. But in the second month, Marcia experienced a hernia at the site of the surgery and was re-admitted to the hospital for surgery to repair the hernia. She experienced a second hernia 2 months later, requiring another surgery. Although the complications caused her to be out of work for 5 months, she took them in stride. "I was happier that I had the complications and not Jason because I could physically take it better than him," she says.

Jason made it through the first few years after transplant without any complications, though, like other organ transplant recipients, he was required to take medications that suppress his immune system to prevent rejection of his new liver. But, in March 2008, his liver enzymes spiked up. He was diagnosed with autoimmune hepatitis, and treated with additional immunosuppressive drugs. Jason continues to be on the drugs, the long-term use of which carries some risk of side effects, such as bone loss. So far, he has tolerated them well and shows no signs of rejecting the transplanted liver.

"It's just something that had to be done. I decided I had to do it," Marcia says, adding "I would hope anyone would do it for someone who was going to die."

Despite the complications along the way, neither Marcia nor Jason regret their decision to undergo what for him has been a life-saving procedure. Jason and his family are grateful to Marcia for donating part of her liver to him, a gift that represents a "huge extension on life." Jason explains that, "She is part of the family. They think the world of her." Marcia feels she has also benefitted from being a living donor. "The benefit is that I know that somebody's alive and that they wouldn't be alive if it wouldn't have been for me. That makes me feel good," she says. When people call her heroic, she responds with her characteristic humanity and resolve. "It's just something that had to be done. I decided I had to do it," she says, adding "I would hope anyone would do it for someone who was going to die."

Helping Others Through Research

Marcia and Jason have also given back to the community of donors and recipients undergoing living donor liver transplantation through their ongoing participation in the A2ALL Study at the Northwestern University site. At the time when they went through their procedures, doctors had limited knowledge about the risk of complications to the donor and recipient. Now, thanks in part to the A2ALL Study's findings, they have additional information that has allowed them to improve the procedure and better inform other donors and recipients of what they can expect, including types and chances of potential complications.

"That's why we got in the study, because we wanted to be able to provide information and to let people know more," says Marcia. Jason echoes that point of view. "I always look at medicine as: we're always practicing it. So the more people that are willing to be in a study so they can get that practice perfect, I'm all for it."

They continue to go to Chicago together every year for Jason's check-ups and to participate in the A2ALL Study. They enjoy reading about the Study's findings and maintain close relationships with the doctors and researchers. "You get to know your whole transplant team, and every time Marcia and I are back there, it's like a reunion," says Jason. "You get another surrogate family."

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Bound for Life

Marcia and Jason are now bound for life—the scars on their abdomens serve as a reminder of their unique connection. Though they no longer work together and live an hour's drive apart, they continue to stay in close contact with each other and their families.

Since the transplant, Marcia has remarried and obtained her RN degree. She now works as an assistant director of nursing at a long-term care and rehabilitation facility. She enjoys spending time with her family, including her 1-year-old grandson, scrap-booking, and traveling the world with her husband. She also remains a strong advocate for living donor liver transplantation, taking advantage of every opportunity to share her experiences with others who are considering becoming living donors, even having "business" cards made with her contact information to distribute to doctors' offices.

Currently, Jason works as the clinical service director for a hospice company, helping care for people at the end of life and their families. He appreciates his new-found abilities post-transplant of renewed energy, eating foods he enjoys, and engaging in physical activity and breaking a sweat without fear of itching. He also enjoys pursuing his interests in photography, deer hunting, and traveling.

No matter where life's journey takes them next, Marcia and Jason share an unbreakable bond, to each other and to making the most out of their lives post-transplant.

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ADULT-TO-ADULT LIVING DONOR LIVER TRANSPLANTATION COHORT STUDY (A2ALL)

The A2ALL Study began in 2000 to study living donor liver transplantation (LDLT), at a time when use of the procedure was expanding despite the limited information available on its risks and benefits compared to the traditional deceased donor liver transplantation (DDLT) procedure. Chief among the study's objectives were to determine whether LDLT was as safe and effective as DDLT for the recipients, and to capture the rates and types of complications from the LDLT surgery, particularly those experienced by the donors. Currently, the Study is conducted by nine liver transplant centers with expertise in adult LDLT located around the country and a central coordinating center.

The Study has already succeeded in showing that LDLT is safe and effective, on par with DDLT, as a life-saving therapy for some patients, particularly those like Jason who would otherwise have to wait a long time for a deceased donor organ. "He is a perfect example of somebody who would have fallen off the waiting list curve," says Dr. Michael Abecassis, Director of the Comprehensive Transplant Center at Northwestern University Feinberg School of Medicine, the A2ALL site where Marcia and Jason's living donor liver transplantation was performed. "Having gone through the Study we can now feel even more confident about doing this type of transplant for this particular individual."

The Study's findings reflect the improvements in LDLT that have taken place in recent years as transplant centers gained more experience in performing the procedure. Dr. James Everhart, the Program Director at the NIDDK who spearheaded the A2ALL Study initiative, recalls, "When living donor liver transplantation first started, the recipients did not do as well. That has changed, and they now do as well as if they had received a liver from a deceased donor." Now, based on the Study's results, having an LDLT rather than a DDLT is advantageous for many patients by reducing their risk of dying while on the waiting list for an organ. "That was an important finding because, for the recipient, there's a real advantage to getting this surgery. It's almost exclusively because they're able to receive a donated organ earlier, rather than waiting for an organ from a deceased donor," says Dr. Everhart.

A2ALL has also achieved its objective to quantify the risk of complications to donors from the LDLT procedure. In a paper published in the *American Journal of Transplantation* and featured elsewhere in this chapter, A2ALL Study investigators described the risks and types of long-term complications for donors undergoing LDLT, in which they found that 40 percent of donors had one or more complications, a rate consistent with other national and international estimates.

"I think one of the best things about A2ALL is that now we can be a little more precise when we are communicating the potential risks, especially to the donor," says Dr. Abecassis. In 2005, when Marcia decided to give part of her liver to Jason, "we really did not have a number to give her, for example, for the risk of a hernia," which she experienced on two occasions. Dr. Abecassis is particularly struck by the willingness of Marcia and others to be living liver donors at a time when

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information about the risk of donor complications was so limited.

Now, because of the long-term efforts of those involved with A2ALL, the picture of LDLT's risks and benefits for donors and recipients is much clearer. "Because the study went on and we were able to follow patients who had the procedure at the beginning of the centers' experiences, we've now been able to say, instead of just a snapshot, here's the movie—this is when you're likely to get these types of complications, this is how severe they're likely to be, and this is how likely it is that those complications might get resolved," says Dr. Abecassis. "We have a lot more confidence now when we talk to potential donors and recipients about what is likely to happen."

Originally designed for 7 years, this highly productive study was funded for an additional 5 years to collect longer-term data on the liver transplant participants and their outcomes. As the Study comes to a close and the investigators consider ways to pursue additional unanswered questions about LDLT, they plan to continue mining the rich resource of long-term data they've collected.

Dr. Abecassis credits the collective power of the Study with enabling its important findings and improving transplant centers' knowledge of LDLT. "Because we were together in a consortium, and we were meeting on an ongoing basis, our program was able to learn certain things from our colleagues, and they learned other things from us. Sometimes, we just learned together from our collective experience. At a time when the procedure was evolving, this was undoubtedly a very valuable exercise." He adds, "This consortium has been particularly productive, not just in terms of academic productivity but in terms of collegial thinking. We're still learning from each other, and this experience continues to benefit our patients."

Additional information on the A2ALL Study can be found online at: www.nih-a2all.org